Saskatchewan

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# FINAL REPORT

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**ISOLATION AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH NECROBACILLOSIS IN WHITE-TAILED DEER**

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# Isolation and Characterization of Bacteria Associated with Necrobacillosis in Farmed Deer.


## Abstract/Summary:

Necrobacillosis is a disease that affects a wide variety of animals, as well as humans, and has been reported worldwide. Extensive studies have been done in bovine, ovine, and caprine subjects. However, despite being recognized as one of the most important diseases affecting deer, no extensive studies have been reported in regards to the presumptive etiological agents of this disease: *Fusobacterium necrophorum* and *Arcanobacterium pyogenes*. The isolation and characterization of these organisms is very important since there have been reported variations in virulence, biotypes and antibiotic sensitivities in strains isolated from conventional farm animals.

Control of the disease requires in depth knowledge of the causative organism(s). This is particularly important in cases of necrobacillosis, which seems to be very difficult to treat with antibacterial drugs, and for which no efficacious chemotheraputic treatment is recognized.

The objective of this study is to isolate the putative agents causing the disease from different farms in Saskatchewan, and to attempt to find common bacterial strains or factors that could enable efficient disease control via vaccination.


## Executive Summary:

The project objective was to define the biological characteristics of the bacteria causing the clinical disease. There are no previous studies in regards to the bacterial strains causing necrobacillosis in farmed deer. In other animal species, it has been found that there exist important variations among strains of *F. necrophorum* and *A. pyogenes*, both in their biological features and in their sensitivities to common antimicrobial drugs.

Characterization of bacteria isolated from the lesions affecting farm deer and its comparison with those isolated from other farms would enable the selection of those strains that might share general characteristics. This selection process would allow for the elaboration of products, such as vaccines, which may protect against similar bacteria originating from different farms.

These studies are needed since in other animals affected by necrobacillosis; there have been reported differences among strains. There has also been detection of organisms, which although relatively similar, have been clearly distinct strains.

It has been reported that *A. pyogenes* and *A. pyogenes*-like (definitely a different group of organisms), as well as different subspecies of *F. necrophorum* have been

established. For example *F. necrophorum* subspecies *necrophorum* and subspecies *funduliforme*. If such differences are encountered among isolates from farmed deer, it might explain the reported failure of vaccinations using commercial bacterins that are intended for use in bovine species, who, would likely be infected with different bacterial strains than those affecting deer.

The antimicrobial sensitivity tests, by comparing a wide range of isolates from different farms, would potentially allow the clinician to select the best antimicrobial drug for the treatment of sick animals.

The results (see technical report for details) show that *A. pyogenes* was present in half of the total number of cases. However, no *A. pyogenes*-like organisms were isolated.

The biochemical characterization of *A. pyogenes* strains demonstrated a rather heterogeneous pattern. Most deer strains were more similar to those isolated from sheep (7 out of 16) than to those from bovine (5 out of 16) sources.

*F. necrophorum* was the anaerobic species most often isolated (28% of cases), followed by *F. varium* (in 19% of cases). Several other species of anaerobic bacteria were also isolated. These results are important since they suggest a possible role for these organisms as etiological agents and should probably be taken into consideration when trying to control the disease by vaccination. These findings might also explain, at least partially, the rather disappointing results of vaccinations with commercial vaccines. The commercial vaccines contain *F. necrophorum* from bovine origin and do not contain *A. pyogenes*. Related to this point, pilot studies using autogenous bacterins have been perceived by the livestock managers/owners to be more effective in disease control than the commercial vaccines. The inclusion of specific strains affecting a specific farm may be the reason for this improved efficacy.

Biochemically, the strains of *F. necrophorum* showed a unique pattern, different from the ones from bovine strains.

Antibiotic resistance was not detected as a problem in either *A. pyogenes* or the anaerobic gram negative bacteria (including *F. necrophorum*). Therefore, failure of chemotherapy is likely related to other factors such as failure of the antibiotics to reach the bacteria due to the type of lesion (e.g. an abscess), and/or inappropriate dose/duration of treatment. This may also be occurring due to delayed detection of the disease.

Genetic studies of the main bacteria (*F. necrophorum, A. pyogenes*, and *F. varium*) are needed to determine if there are any common virulence factors or strains that can have future use in a "general" vaccine for deer.

**Practical Recommendations:**

At the moment, it seems that vaccination with autogenous bacterins is the most promising way to control necrobacillosis. However, data proving the efficacy of such a

practice cannot be obtained with the results we have so far. Methodic epidemiological studies including elaborate questionnaires aimed to prove the efficacy of these products are needed.

Early treatment seems to be critical, so constant observation of the animals is needed to detect cases before lesions become too advanced. Treatment should include drugs that are efficient for both gram negative anaerobes and *A. pyogenes*.

**Technical report:**

Objective: The isolation and characterization of bacteria associated with necrobacillosis in deer. Specific objectives are described in the executive summary.

Activities: Processing of samples taken from animals with necrobacillosis from different farms. 32 cases were received from clinical cases of deer raised on different farms from February 2000 to January 2001.

Samples were processed to culture aerobic and anaerobic bacteria, and antibiotic sensitivity tests were done on the most common isolates.

Detailed biochemical characterization was performed on these organisms using conventional tests and well as commercial kits.

Results:

Biochemical Characterization: *A. pyogenes* strains were quite heterogeneous, although more similarity was found between deer and ovine strains than between deer and bovine strains. No *A. pyogenes*-like strains were identified (although there has been some identified in the bovine studies).

Anaerobic Bacteria: The following species were isolated: *F. necrophorum* (9 strains), *F. varium* (6 strains), *B. oralis* (2 strains), *B. capillosum* (2 strains), *B. uniformis* (1 strain), and *B. melaninogenicus* (1 strain). Again, the *F.necorphorum* strains were notably different from those of bovine origin.

The results of the antibiotic sensitivity tests (MIC µg/ml) for *A. pyogenes* are as follows:

| Antibiotic | For 90% of isolates |
|---|---|
| Ampicillin | 0.023 |
| Ampicillin/Sulbactam | 0.023 |
| Gentamicin | 3.0 |
| Enrofloxacin | 2.0 |
| Erythromycin | 0.016 |
| Penicillin G | 0.016 |
| Tetracycline | 0.19 |

| | |
|---|---|
| Lincomycin | 0.12 |
| Clindamycin | 0.5 |
| Florfenicol | 0.5 |

For the gram negative anaerobes, including *F. necrophorum*, the MIC µg/ml results were:

| Antibiotic | For 90% of isolates |
|---|---|
| Ampicillin | 0.75 |
| Ampicillin/Sulbactam | 0.50 |
| Amoxicillin/Clavulanic Acid | 0.19 |
| Enrofloxacin | 4.0 |
| Penicillin G | 0.19 |
| Tetracycline | 0.19 |
| Clindamycin | 4.0 |

**Discussion and Conclusions:**

*A. pyogenes* was isolated in a very large proportion of cases, although no specific biochemical pattern was detected. The inclusion of this organism, in vaccines against necrobacillosis in deer is therefore highly recommended.

Antibiotic resistance is not a problem with the bacteria involved in necrobacillosis in deer. More attention is required to other factors such as treatment dose and duration, early detection of cases, and management measures.

*F. necrophorum* strains presented a rather uniform biochemical pattern, differing from that of the bovine strains. *Fusobacterium* spp. other than *F. necrophorum* probably play an important role in the disease. All anaerobic bacteria isolated from clinical cases are to be included in the production of autogenous bacterins (together with *A. pyogenes*).

**Further Developments:**

It is necessary to scientifically assess the benefits of autogenous bacterins. An epidemiological study is strongly recommended.

The pathogenesis of the disease in deer is not well understood, so studies dealing with management factors, and/or associated infections (viral??) triggering necrobacillosis are also needed.

**Information of Benefit to Producers, Processors and Government:**

Necrobacillosis is probably the main infectious disease affecting deer farms. This study confirmed its extensive presentation on deer farms in the province. The confidentiality offered to the producers allowed the determination of the magnitude of the problem.

Antibiotic resistance is not a problem, however for a treatment to be efficient, antibiotic treatment should be initiated as early as possible with antibiotics that achieve good tissue concentrations. Care should be taken to ensure treatment is followed as long as necessary; veterinary advice is strongly recommended to the owners.

In short term, the use of autogenous bacterins is recommended. Studies should be done to select specific strains and/or subunit vaccines containing adequate antigens to elicit good protective immunity in the vaccinated animal.

**Personnel:**

Professional: Drs. M. Chirino-Trejo and M. Woodbury - salaried professors at WCVM

Technical: Fei Huang - part time microbiological lab technician - salary plus employment costs total = $ 15,229.31

**Equipment:**

N/A

**Project Developed Materials:**

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N/A

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